

Remarks

Claims 1-7 and 19-25 are presently pending in the subject application. By this Amendment, the applicants have amended claim 2 to comply with 37 C.F.R. §1.825. The undersigned avers that no new matter is introduced by this Amendment. Entry and consideration of the amendments presented herein is respectfully requested. Accordingly, claims 1-7 and 19-25 are currently before the Examiner for consideration. Favorable consideration of the pending claims is respectfully requested.

As an initial matter, a Revocation and New Power of Attorney was previously submitted to the Patent Office by facsimile on October 25, 2002. Enclosed is a copy of the previously submitted Revocation and New Power of Attorney for the Examiner's convenience.

The applicants gratefully acknowledge the Examiner's careful review of the subject specification. By this Amendment, the applicants have renumbered the sequence identifiers, replaced the Brief Description of the Drawings section, and added a Brief Description of the Sequences section in order to facilitate compliance with 37 C.F.R. §1.821-1.825. SEQ ID NOs: 8 and 9 of the previous sequence list (described at page 62 of the specification) were identical to SEQ ID NOs: 4 and 5 (described at page 59) of the specification; therefore, the applicants have renumbered the sequence identifiers to avoid redundancy. The applicants have also added an ATP-binding motif (new SEQ ID NO: 13) to the sequence list, which is described at page 7 of the specification. New SEQ ID NOs: 4-7 are now correctly designated as "artificial sequences" (primers). The descriptions of Figures 42, 43, and 44 now include references to numeric sequence identifiers (new SEQ ID NOs: 10, 11, and 12, respectively).

A Submission of Sequence Listing and Statement under 37 C.F.R. §1.821 accompany this Amendment.

In view of the foregoing remarks and amendments, the applicants believe that the currently pending claims are in condition for allowance, and such action is respectfully requested.

The Commissioner is hereby authorized to charge any fees under 37 C.F.R. §§ 1.16 or 1.17 as required by this paper to Deposit Account 19-0065.

The applicants invite the Examiner to call the undersigned if clarification is needed on any of this response, or if the Examiner believes a telephonic interview would expedite the prosecution of the subject application to completion.

Respectfully submitted,



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Attachments: Petition and Fee for Extension of Time
Copy of Communication with accompanying Revocation and New Power of Attorney
Submission of Sequence Listing and Statement Under 37 C.F.R. §1.821
CRF and Paper Copies of Sequence Listing
Copy of Notice to Comply
Marked-up Version of Amended Claim
Marked-up Version of Substitute Paragraphs



Marked-Up Version of Amended Claims

Claim 2(amended):

The construct of claim 1 wherein the metabolite responsive instability element comprises the sequence TAACTCTGAATTTTAAAACCCGAAGTCAAGAGCTAGTA [~~SEQ ID NO: 11~~] of SEQ ID NO: 9.

Marked-up Version of Substitute Paragraphs**Brief Description Of The Drawings**

- Figure 1.** Schematic representation of the PKC isozymes domain structure
- Figure 2.** A schematic representation of PKC β sequence as deduced from the cDNA analysis
- Figure 3.** Alternative splicing of PKC β pre-mRNA
- Figure 4.** PKC β II mRNA is generated via exon inclusion in the alternative splicing of PKC β
- Figure 5.** Generation of PKC activators
- Figure 6.** Activation of PKC by diacylglycerol and Ca²⁺
- Figure 7.** Model for activation of PKC
- Figure 8.** Localization of PKC isozymes by anchoring proteins
- Figure 9.** Arterial smooth muscle cell phenotypes
- Figure 10.** Representation of the response-to-injury hypothesis of atherosclerosis proposed by Ross
- Figure 11.** The MAP kinase pathway transduces the signal from the membrane to the nucleus
- Figure 12.** The role of phosphoinositide 3-kinase (PI3 kinase) in signal transduction
- Figure 13.** Levels of eukaryotic gene regulation
- Figure 14.** The fate of mRNA in mammalian cells
- Figure 15.** Representation of the structural elements involved in regulating mRNA stability
- Figure 16.** PKC β I protein levels remain unaltered in the presence of high glucose
- Figure 17.** PKC β II protein levels decreased by 55% in the presence of glucose
- Figure 18.** Down regulation of PKC β (I+II) mRNA by glucose
- Figure 19.** Map of PKC β promoter and lengths of deletion constructs
- Figure 20.** Effect of high glucose on PKC β deletion constructs
- Figure 21.** Construct D quenches PKC β promoter activity at 10h post-synchronization corresponding to S phase
- Figure 22.** Northern blot analysis of PKC β II mRNA in A10 cells treated with actinomycin D in the presence or absence of high glucose
- Figure 23.** *In vitro* assay for RNA stability

- Figure 24.** *In vitro* assay for RNA stability in the presence of EDTA
- Figure 25.** A schematic representation of the PKC β as deduced from cDNA sequence analysis
- Figure 26.** High glucose destabilizes PKC β II mRNA
- Figure 27.** PKC β II cDNA (350 bp) sequence (SEQ ID NO: 8)
- Figure 28.** PKC β II cDNA (350 bp) restriction sites map
- Figure 29.** Effect of glucose and insulin on PKC β II mRNA in aorta smooth muscle cells
- Figure 30.** Glucosamine does not affect PKC β II mRNA stability
- Figure 31.** Effect of glucose metabolites on PKC β II mRNA stability
- Figure 32.** Effect of cycloheximide on glucose-induced PKC β II destabilization
- Figure 33.** RT-PCR analysis of PKC β I and β II mRNA after okadaic acid treatment
- Figure 34.** The p β G-PKC β II chimeric minigene
- Figure 35.** The p β G-PKC β II chimeric minigene is destabilized by high glucose
- Figure 36.** Half-life analysis of p β G-PKC β II mRNA
- Figure 37.** Schematic of the RNA probes used for the RNA EMSAs
- Figure 38.** RNA electrophoretic mobility shift assay using full length PKC β II mRNA probe
- Figure 39.** A cytosolic factor binds to a glucose-regulated element present in the PKC β II coding region
- Figure 40.** No RNA protein binding observed using RNA probe C
- Figure 41.** UV cross-linking analysis of the RNA-protein binding complex
- Figure 42.** PKC β II mRNA sequence (SEQ ID NO: 10) and RNA secondary structure analysis
- Figure 43.** PKC β II mRNA sequence linearized at 175 bp with *Bgl* II (SEQ ID NO: 11) and RNA secondary structure analysis
- Figure 44.** PKC β II mRNA sequence linearized at 137 bp with *Hpa* I (SEQ ID NO: 12) and RNA secondary structure analysis
- Figure 45.** Diagram of 3' exons encoding PKC β I and PKC β II via exon inclusion/exclusion and regions of PKC β II exon involved in ~~destabilization~~ destabilization of mRNA.
- Figure 46.** Restriction map of 3' ~~region of~~ region of PK β II exon with portions of 3' and 5' flanking exons to be cloned downstream of the cDNA of interest.